Anti-inflammatory potential of *Antrodia Camphorata* through inhibition of iNOS, COX-2 and cytokines via the NF-κB pathway

You-Cheng Hseu a, Fang-Yang Wu b, Jia-Juan Wu d, Jing-Yi Chen d, Wen-Huei Chang c, Fung-Jou Lu c, Yu-Ching Lai d, Hsin-Ling Yang d,*

aDepartment of Food Science, Changtai Institute of Health Sciences and Technology, Taiwan  
bInstitute of Environmental Health, China Medical University, Taiwan  
cSchool of Applied Chemistry, Chung Shan University, Taiwan  
dInstitute of Nutrition, China Medical University, 91 Hsueh Shih Road, Taichung 40421, Taiwan

Received 14 January 2005; received in revised form 23 February 2005; accepted 20 June 2005

Abstract

*Antrodia camphorata* (*A. camphorata*), well known in Taiwan as a traditional Chinese medicine, has been shown to exhibit antioxidant and anticancer effects. In the present study, therefore, we have examined the effects of the fermented culture broth of *A. camphorata* (25–100 μg/ml) in terms of lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin E2 (PGE2) production, and inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in RAW 264.7 macrophages. Our results indicate concentration-dependent *A. camphorata* inhibition of LPS-induced NO and PGE2 production, without appreciable cytotoxicity on the RAW 264.7 cells. *A. camphorata* also attenuates the production of LPS-induced tumor necrosis factor (TNF-α) and interleukin (IL)-1β. Furthermore, *A. camphorata* blocks the IκB-α degradation induced by LPS. These results indicate that *A. camphorata* inhibits LPS induction of cytokine, iNOS and COX-2 expression by blocking NF-κB activation. Therefore, we report the first confirmation of the anti-inflammatory potential of this traditionally employed herbal medicine in vitro.

© 2005 Elsevier B.V. All rights reserved.

Keywords: *Antrodia camphorata*; Cytokines; Inducible nitric oxide synthase (iNOS); Cyclooxygenase 2 (COX-2); Nuclear factor-κB (NF-κB)

1. Introduction

*Antrodia camphorata*, a new basidiomycete in the Polyporaceae (Aphyllophorales), which causes brown heart rot in *Cinnamomum kanehirai* hay (Lauraceae) in Taiwan, has been identified as a new genus of the *Antrodia* species [1,2]. The *A. camphorata* fungus is rare and expensive as it grows only on the inner heartwood wall of the *C. kanehirai* and cannot be cultivated. It has been utilized in traditional Chinese medicine for the treatment of food and drug intoxication, diarrhea, abdominal pain, hypertension, skin itches,
and liver cancer [3], however, very few biological activity tests are reported.

Macrophages play an important role in inflammatory disease through the release of factors such as nitric oxide (NO), prostaglandin mediators, and cytokines involved in the immune response [4–6]. Production of these macrophage mediators has been determined in many inflammatory tissues, along with increased expression of their mRNAs, following exposure to immune stimulants including bacterial endotoxin lipopolysaccharide (LPS). It has been demonstrated that NO is an important regulatory molecule for diverse physiological functions such as vasodilation, neural communication, and host defense [7,8]. NO is a free radical generated through the conversion of L-arginine to citrulline, catalysed by NO synthase (NOS). Molecule cloning and sequencing analysis have revealed at least three types of NOS isoforms existing in cells [9–11]. NOS isoymes that appear in the vascular endothelium (eNOS) and central and peripheral neurons (nNOS) are constitutive (cNOS). Release of NO catalyzed by cNOS plays a role in maintaining active vasodilation through a Ca²⁺-dependent pathway. On the other hand, NOS in macrophages and hepatocytes is inducible, and its activation is Ca²⁺-independent. The induced iNOS catalyzes the formation and release of a large amount of NO, which then plays a key role in disease pathophysiology [12,13]. Cyclooxygenase (COX) is the enzyme catalyzing the conversion of arachidonic acid to prostaglandin H₂, the precursor of a variety of biologically active mediators, such as PGE₂, prostacyclin, and thromboxane A₂ [14,15]. Two forms of this enzyme have been identified: COX-1, a constitutive cyclooxygenase, and COX-2, an isoform induced in response to many stimulants and activated at the site of the inflammation [16–18]. COX-2, which is rapidly induced in macrophages and endothelial cells by proinflammatory cytokines, may be responsible for the edema and vasodilation associated with inflammation. Overproduction of the inflammatory mediators involves many diseases, such as rheumatoid arthritis, chronic hepatitis, and pulmonary fibrosis [19–21]. Thus, inhibition of the production of these inflammatory mediators may prevent or suppress a variety of inflammatory diseases, including sepsis, and endotoxemia.

Nuclear factor-κB (NF-κB), a nuclear transcription factor, regulates the expression of various genes, including cytokines, iNOS and COX-2, that play critical roles in apoptosis, tumorigenesis, various autoimmune diseases, and inflammation [22]. NF-κB exists in most cells as homodimeric or heterodimeric complexes of p50 and p65 subunits and remains inactive in the cytoplasm of cells associated with the NF-κB inhibitory protein (I-κB) [23]. NF-κB is activated in response to various inflammatory stimuli including bacterial LPS and LPS induced NF-κB activation through increasing nuclear p65 protein associated with decreased cytosolic IκB protein [23]. Because of its ubiquitous role in the pathogenesis of inflammatory gene expression, NF-κB is a current target for treating various diseases [24,25].

For more than 2000 years, medicinal mushrooms have been used in China to improve health and achieve longevity. A. Camphorata has recently become popular as a drug remedy in Taiwan as well as a source of physiologically beneficial mushrooms. A number of reports have indicated that A. Camphorata possess antioxidant, antitumour and immunomodulating activities [26–31]. In our previous study, A. camphorata was used for the inhibition of AAPH-induced oxidative hemolysis and lipid/protein peroxidation in normal human erythrocytes [32]. Interestingly, A. camphorata exhibits significant apoptotic effects against leukemia HL-60 cells, but not against cultured human endothelial cells, suggesting that A. camphorata may possess protective antioxidant and anticancer properties [33]. The aim of this research was, therefore, to investigate potential anti-inflammatory properties of the fermented broth of A. camphorata harvested from submerged cultures on lipopolysaccharide (LPS)-induced cytokines, such as TNF-α and IL-1β, NO and PGE₂ production, and expression of iNOS and COX-2 through blockade of NF-κB activation in RAW264.7 macrophages. The murine macrophage cell line RAW264.7 was used for this purpose. Bacterial lipopolysaccharides served as a stimulus for these established models of infection and inflammation.

2. Materials and methods

2.1. Materials

Cell-culture medium (DMEM), fetal calf serum, and penicillin/streptomycin were obtained from
Gibco/BRL Life Technologies Inc. (Eggenstein, Germany). Anti-COX-2 polyclonal antibody was purchased from Santa Cruz (Heidelberg, Germany); anti-NOS antibody was obtained from Santa Cruz (Heidelberg, Germany). All other materials were purchased from Sigma Chemical Co (Deisenhofen, Germany).

2.2. Preparation of the fermented culture broth of A. camphorata

The A. camphorata culture was inoculated on potato dextrose agar and incubated at 30 °C for 15–20 days. The whole colony was then cut and put into a flask with 50-ml sterile water. After homogenization, the fragmented mycelial suspension was used as an inoculum. The seed culture was prepared in a 20-L fermentor (BioTop) agitated at 150 rpm with an aeration rate of 0.2 vvm at 30 °C. A 5-day culture of 15 L of mycelia inoculum was inoculated into a 250-L agitated fermentor (BioTop). The fermentation conditions were the same as for the seed fermentation, but aeration rate was 0.075 vvm. The fermentation product was then harvested at hour 331 and poured through a non-woven fabric on a 20-mesh sieve to separate the deep-red fermented culture broth and the mycelia, and then centrifuged at 3000 g for 10 min followed by passage through a 0.2-μm pore-size filter. The culture broth was then concentrated under vacuum and freeze-dried to powder form. The yield of dry matter from the culture broth was 9.72 g/L. For the preparation of aqueous solution, the powder samples were solubilized with 10-mM sodium phosphate buffer (pH 7.4) containing 0.15-M sodium chloride (PBS) at 25 °C. The stock solution was stored at −20 °C before analysis of its anti-inflammatory properties.

2.3. Cell culture and assessment of cell viability

The murine macrophage cell line RAW 264.7 (American Type Culture Collection ATCC, TIB 71, Rockville, MD) was cultured in DMEM containing 4-mM glutamine and 10% heat-inactivated fetal calf serum and incubated with A. camphorata (0, 25, 50, and 100 μg/ml) in the presence or absence of LPS (1 μg/ml) for 18 h. One hundred microliters of each supernatant was mixed with the same volume of Griess reagent (0.75% sulfanilamide in 0.5 M HCl and 0.075% naphthylethylene diamine dihydrochloride in water); absorbance of the mixture at 540 nm was determined using an ELISA plate reader (Dynatech MR-7000; Dynatech Laboratories).

2.4. Nitrite assay

The concentration of NO in culture supernatants was determined as nitrite, a major stable product of NO, by the Griess reagent as described previously [34]. The RAW 264.7 cells were plated in a 12-well cell-culture dish and incubated with A. camphorata (0, 25, 50, and 100 μg/ml) in the presence or absence of LPS (1 μg/ml) for 12, 18, and 24 h. One hundred microliters of each supernatant was mixed with 100 μl of the Griess reagent (0.1% naphthylethylene diamine dihydrochloride in water); absorbance of the mixture at 540 nm was determined using an ELISA plate reader (Dynatech MR-7000; Dynatech Laboratories).

2.5. Measurement of PGE2 production

RAW 264.7 cells were subcultured in 12-well plates and incubated with A. camphorata (0, 25, 50, and 100 μg/ml) in the presence or absence of LPS (1 μg/ml) for 18 h. One hundred microliters of culture-medium supernatant was collected for determination of PGE2 concentration by ELISA (Cayman Enzyme Immunoassay Kit).

2.6. Western blot analysis

RAW 264.7 cells were incubated with A. camphorata (0, 25, 50, and 100 μg/ml) in the presence or absence of LPS (1 μg/ml) for 18 h. Total cellular extracts were prepared according to Muller et al., [35], separated on SDS-polyacrylamide minigels (8% for iNOS/COX-2, and 10% for IR:B) and transferred to Immobilon polyvinylidene difluoride membranes (PerkinElmer Life Sciences, Inc. Boston) [36]. Protein concentration was determined using the Bio-Rad protein assay kit. The membranes were blocked with 5% nonfat milk in PBS-Tween (0.1%) for 10 min
and then incubated with antibody for mouse iNOS, COX-2, IkB (Santa Cruz Biotechnology, Inc.), or β-actin (Sigma, Deisenhofen, Germany) in PBS-Tween containing 1% nonfat milk for 2 h at room temperature. After washing three times with PBS-Tween, the membrane was hybridized with secondary antibody conjugated with horseradish peroxidase for 2 h at room temperature. Following further washing in PBS-Tween, immunoreactive bands were visualized using ECL detection system (Amersham Corp.).

2.7. Assay of TNF-α and IL-1β

Levels of TNF-α and IL-1β were measured in cell culture media using ELISA kits (R and D Systems, Minneapolis, MN). The macrophage cells were plated in a 6-well cell-culture dish at a density of \(8 \times 10^5\) cells/well and incubated with \(A.\) camphorata (0, 25, 50, and 100 \(\mu\)g/ml) in the presence or absence of LPS (1 \(\mu\)g/ml) for 18 h. The medium was collected and assayed for TNF-α and IL-1β using the ELISA kits. For TNF-α, the medium was diluted with Reagent Diluent at 1:5 ratio; 100 \(\mu\)l of each diluted sample was used for ELISA. Quantitation of the ELISA results was performed using a Microplate Spectrophotometer (Bio-Tek Instruments, ICN, USA) set to a wavelength of 450 nm and corrected for absorbance at 540 nm, according to the manufacturer’s instruction.

2.8. Measurement of ROS generation by chemiluminescence assay

ROS production was determined according to the method of Lu et al., [37] with some modification. Briefly, cells were suspended in DMEM/10% FCS and incubated with \(A.\) camphorata (0, 25, 50, and 100 \(\mu\)g/ml) in the presence or absence of LPS (1 \(\mu\)g/ml) for 18 h. LPS has been shown to cause an increase cellular ROS production [38]. After trypsinization, the cells were washed, resuspended at \(4 \times 10^5\) cells/ml, and then placed in a dark chamber containing luminol (1 mM), and light emission measured using an ultra-sensitive chemiluminescence detector (model CLD-110; Tohoku Electronic Industrial Co., Sendai, Japan) at 10-s intervals for a total of 10 min. Luminol-derived chemiluminescence has been used to assess the generation of reactive oxygen species and free radical intermediates in biological system [39]. The total chemiluminescence intensity was calculated by integrating the area under the curve minus the background level, which was equal to the dark average, and the results expressed as counts per 10 s.

2.9. Statistics

Mean data values are presented with their deviation (mean ± SD). Analysis of variance (ANOVA) was used for all data analysis, followed by Dunnett’s test for pairwise comparison. Statistical significance was defined as \(P<0.05\).

3. Results

In this study, the murine macrophage cell line RAW 264.7 was used to investigate potential anti-inflammatory properties of the fermented culture broth of \(A.\) camphorata (harvested from submerged cultures) on LPS-induced cytokines, NO and PGE2 production, and iNOS and COX-2 expression by blocking NF-κB activation in vitro.

3.1. Effects of \(A.\) camphorata on LPS-induced NO production

RAW 264.7 macrophages were stimulated with LPS (1 \(\mu\)g/ml) for 12, 18, and 24 h to evoke NO synthesis. NO was measured as the accumulation of its stable metabolite, nitrite, in the supernatant. Coincubation of cells with the fermented culture broth of \(A.\) camphorata (25–100 \(\mu\)g/ml) and LPS resulted in a significant, dose and time-dependent reduction of NO production (Table 1). Unstimulated macrophages, after 18 h of incubation in culture medium, produced background levels of nitrite (Table 1). When the cells were incubated with \(A.\) camphorata (25–100 \(\mu\)g/ml) alone, the concentration of nitrite in the medium was maintained at a background level similar to that in the unstimulated samples (Table 1).

3.2. Effects of \(A.\) camphorata on the viability of RAW 264.7 macrophages

To test whether \(A.\) camphorata affects RAW 264.7 macrophages, the viability of cultured RAW 264.7
cells was examined. RAW 264.7 cell numbers after 18 h of incubation were not affected by *A. camphorata* at 0, 25, and 50 µg/ml in the presence or absence of LPS (1 µg/ml) (Fig. 1), however, there was a decrease in cell number at 100 µg/ml.

### 3.3. Effects of *A. camphorata* on LPS-induced PGE₂ production

PGE₂ represents the most important inflammatory product of COX-2 activity and, thus, it was quantified in the supernatant. RAW 264.7 macrophages were stimulated with LPS (1 µg/ml) for 18 h. Coincubation of the cells with *A. camphorata* (25–100 µg/ml) and LPS resulted in a significant, dose-dependent reduction in PGE₂ production (Fig. 2). Unstimulated macrophages, after 18 h of incubation in culture medium, produced background levels of PGE₂. When the cells were incubated with *A. camphorata* (100 µg/ml) alone, the concentration of PGE₂ in the culture medium was maintained at a background level similar to that in the unstimulated samples (Fig. 2).

### 3.4. Effects of *A. camphorata* on the expression of iNOS and COX-2 protein

In order to determine the mechanisms by which *A. camphorata* reduces LPS-induced NO and PGE₂ production, we studied the ability of *A. camphorata* (25–100 µg/ml) to influence the LPS-induced expression of iNOS or COX-2 protein. Western blot experiments showed induction of iNOS and COX-2 protein in

![Graph showing cell viability of RAW 264.7 macrophages](image-url)
RAW 264.7 cells after 18 h LPS (1 µg/ml) treatment (Fig. 3). This expression was markedly attenuated in cells co-treated with *A. camphorata* (Fig. 3).

### 3.5. Effects of *A. camphorata* on LPS-induced TNF-α and IL-1β production

To determine the potential effects of *A. camphorata* on the production of proinflammatory cytokines such as TNF-α and IL-1β, the RAW 264.7 cells were incubated with *A. camphorata* (0, 25, 50, and 100 µg/ml) in the presence or absence of LPS (1 µg/ml) for 18 h, and the cytokine levels were measured in the culture media by ELISA. The TNF-α and IL-1β levels were increased in the culture media of LPS-stimulated RAW 264.7 cells, and these increases were significantly decreased in a concentration-dependent manner by treatment with *A. camphorata* (Fig. 4A and B).

### 3.6. Inhibition of NF-κB activation by *A. camphorata*

To assess the effect of *A. camphorata* (0–100 µg/ml) on the early stages of cytokine, iNOS and COX-2 expression, the activation of NF-κB in RAW 264.7 macrophages in response to LPS was examined (Fig. 5). The heteromeric NF-κB complex is sequestered in the cytoplasm as an inactive precursor complexed with an inhibitory protein, an IκB-like protein, and LPS induced NF-κB activation through increasing nuclear p65 protein associated with decreased cytosolic IκB protein. As shown in Fig. 5, incubation of RAW 264.7 macrophages with 1 µg/ml of LPS resulted in decreased cytosolic IκB protein. This phenomenon was inhibited significantly by *A. camphorata* (Fig. 5). Our results show that *A. camphorata* treatment blocks the degradation of IκB and, therefore, NF-κB activation induced by LPS in RAW 264.7 cells.

### 3.7. Effects of *A. camphorata* on LPS-induced ROS production

To assess the effect of *A. camphorata* on LPS-induced ROS production, the RAW 264.7 cells were incubated with *A. camphorata* (0, 25, 50, and 100 µg/ml) in the presence or absence of LPS (1 µg/ml) for 18 h. LPS has been shown to cause an increase cellular ROS production. The production of ROS can be detected by using an ultrasensitive chemiluminescence analyzer, which monitors the emission of chemiluminescence derived from the energy of a chemical reaction. Luminol is typically used as a chemiluminigenic probe for estimating ROS generation. As shown in Fig. 6, the LPS-stimulated RAW 264.7 cells show an increase in ROS production. Coincubation of cells with *A. camphorata* (25–100 µg/ml) and LPS resulted in a significant reduction of...
ROS production (Fig. 6). Unstimulated macrophages, after 18 h of incubation, produced background levels of ROS. When the cells were incubated with *A. camphorata* (100 µg/ml) alone, the concentration of ROS was maintained at a background level similar to that in the unstimulated samples (Fig. 2).

4. Discussion

The present study was undertaken to elucidate the pharmacological and biological effects of the fermented broth of *A. camphorata* on the production of inflammatory mediators in macrophages. The results indicate that *A. camphorata* is an effective inhibitor of LPS-induced cytokines, such as TNF-α and IL-1β, NO and PGE₂ production, and expression of iNOS and COX-2 through blockade of NF-κB activation in RAW 264.7 macrophages. *A. camphorata* appears to be a potential therapeutic agent for treating LPS-induced sepsis syndrome. It has also been determined that the anti-inflammatory activity of the culture medium is lower than that of *A. camphorata* in submerged culture (data not shown), indicating that anti-inflammatory components of the fungus must be derived from secondary metabolites of the mycelia. In this study, except for 100 µg/ml of *A. camphorata* inhibiting of the LPS-stimulated cell growth, no other concentration of *A. camphorata* showed any significantly cytotoxicity to LPS-treated cells, suggesting that the NO and PGE₂ inhibitory effects of *A. camphorata* were not due to cell death.

Fig. 3. Effects of *A. camphorata* on the expression of iNOS and COX-2 proteins in RAW 264.7 macrophages. The RAW 264.7 cells were incubated with *A. camphorata* (0, 25, 50, and 100 µg/ml) in the presence or absence of LPS (1 µg/ml) for 18 h. Protein (50 µg) from each sample was resolved on 10% SDS-PAGE, and western blotting performed. β-actin was used as a control. Typical result from three independent experiments is shown. *Statistically significant difference compared to LPS-activated cells (P<0.05). #Statistically significant difference compared to untreated cells (P<0.05). AC, *A. camphorata*.
NO and prostaglandins, which are produced by iNOS and COX-2, respectively, have been implicated as important mediators in the processes of inflammation [40]. Our results demonstrate that *A. camphorata* inhibits LPS-induced NO and PGE₂ production in a concentration-dependent manner in RAW 264.7 macrophages. This suppression was correlated with down-regulation of iNOS and COX-2 expression. It has been demonstrated that NO plays a pivotal role as neurotransmitter, vasodilator, and immune regulator in a variety of tissues at physiological concentrations [7]. However, high levels of NO produced by iNOS have been defined as a cytotoxic molecule in inflammation and endotoxemia [41]. Like NO, PGE₂ is a pleiotropic mediator produced at inflammatory sites by COX-2 that gives rise to pain, swelling and stiffness [42]. Thus, potential inhibitors of iNOS and COX-2 have been considered.

Fig. 4. Effects of *A. camphorata* on LPS-induced TNF-α and IL-1β production in RAW 264.7 cells. The RAW 264.7 cells were incubated with *A. camphorata* (0, 25, 50, and 100 µg/ml) in the presence or absence of LPS (1 µg/ml) for 18 h. The extracellular levels of TNF-α (A) and IL-1β (B) were measured in culture media using commercial ELISA kits. Results are presented as the mean ± SD of at least three independent experiments. *Statistically significant difference compared to LPS-activated cells (P<0.05). #Statistically significant difference compared to untreated cells (P<0.05). AC, *A. camphorata*.
effective therapeutically for preventing inflammatory reaction and disease.

Cumulative evidence indicates that an abnormality in the production or function of cytokines, such as TNF-α and IL-1β, plays an essential role in many inflammatory lesions [43]. Inflammatory stimuli, such as LPS, induce cytokines in the process of macrophage activation, which mediates tissue responses in
different phases of inflammation in a sequential and concerted manner [44]. Exposure to LPS causes inflammatory liver damage and septic shock due to production of high levels of these cytokines [45]. Thus, inhibition of cytokine production or function serves as a key mechanism in the control of inflammation [46]. In congruence with these results, we found that *A. camphorata* inhibits TNF-α and IL-1β in RAW 264.7 cells stimulated by LPS. These findings provide evidence that *A. camphorata* may possess potential anti-inflammatory activity.

It has been shown that NF-κB activation is a factor critical to expression of various cytokines, and iNOS and COX-2 in macrophages in response to LPS [22,23]. The heteromeric NF-κB complex is sequestered in the cytoplasm as an inactive precursor complexed with an inhibitory protein, an IκB-like protein, and LPS induced NF-κB activation through increasing nuclear p65 protein associated with decreased cytosolic IκB protein [23]. These findings suggest that *A. camphorata* treatment blocks the degradation of IκB and, therefore, NF-κB activation induced by LPS in RAW 264.7 macrophages. In this study, we describe novel anti-inflammatory mechanisms mediated by *A. camphorata*, which are based on the inhibition of LPS-mediated activation of NF-κB.

It has been shown that several natural antioxidant compounds directly inhibit the expression of NF-κB-dependent cytokines, iNOS and COX-2 and, thus, reduce inflammation [47–49]. The suppressive effects of these antioxidant compounds on the production of the associated inflammatory mediators are associated with their antioxidant activities. The antioxidant NF-κB inhibitors restrict production of inflammatory mediators through suppression of their gene expression and also prevent inflammatory diseases. These results demonstrate that *A. camphorata* inhibits LPS-mediated ROS production in RAW 264.7 macrophages. Our previous study suggests that *A. camphorata* may possess protective antioxidant properties [32]. Thus, potential inhibition of ROS generation induced by *A. camphorata* is in accord with inhibition of NF-κB-dependent cytokines, iNOS and COX-2 expression and, thus, reduce inflammation.

Herbal remedies have a long tradition in disease treatment, especially in eastern medicine. Active components, such as polysaccharides, triterpenoids, and polyphenols, in medicinal mushrooms and plants as well as edible analogues, possess various pharmacological properties, along with antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities [50–54]. Compounds isolated from *A. camphorata* include polysaccharides, ergostan-type triterpenoids, a sesquiterpene, and phenyl and biphenyl derivatives [55,56]. A previous study, based on evaluations of different antioxidant test systems, has demonstrated an association between the antioxidant activity of *A. camphorata* and its polyphenol, triterpenoid and polysaccharide contents [26]. The reported yields of the fermented *A. camphorata* broth in terms of polysaccharides, crude triterpenoids and total polyphenols were 23.2%, 67, and 47 mg/g, respectively [26]. In contrast, no polysaccharides, crude triterpenoids, or total polyphenols were detected in the dry matter of the culture medium [26]. It appears reasonable to suggest, therefore, that *A. camphorata* metabolizes the culture medium and releases active components, such as polysaccharides, polyphenols and triterpenoids, during the fermentation process of the submerged culture. The results imply that, at higher contents, natural polysaccharides, triterpenoids, and polyphenols possibly act as anti-inflammatory agents, rendering them the most-effective fraction of the *A. camphorata* extract. However, characterization of candidate compounds that may account for the anti-inflammatory activity of *A. camphorata* requires further study.

In summary, to the best of our knowledge this study is the first to show that the fermented broth of *A. camphorata* inhibits the production of cytokines, NO and PGE2 in LPS-stimulated macrophages. This anti-inflammatory effect occurs by down-regulation of iNOS and COX-2 expression via the suppression of NF-κB activation in the pathophysiology of inflammatory disease. This anti-inflammatory potential may, therefore, indicate the presence of a novel mechanism of action underlying the apparent efficacy of this traditionally employed herbal medicine. However, further in vivo investigation of this activity is necessary to elaborate the mechanism/s and permit full exploitation of its promise.

**Acknowledgment**

This work was supported by grants NSC93-2313-B-039-002 and CMU 93-NT-08 from the National...
Science Council and China Medical University of the Republic of China. *A. camphorata* was provided by the Food Industry Research and Development Institute in Taiwan.

References

[34] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982;126:131 – 8.


